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EFFECT OF DIETARY INCLUSION LEVELS OF *Moringa olerifera* OIL ON THE GROWTH PERFORMANCE AND NUTRIENT RETENTION OF BROILER STARTER CHICKS

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ABSTRACT

An experiment was carried out to examine the effect of dietary inclusion of Moringa oleifera oil (MOO) on the growth performance and nutrient retention of broiler chicks. A total of 180-day-old broiler chicks (Arbor acre) of mixed sex were randomly allotted into six dietary treatments of 30 birds per treatment; each treatment was further divided into 3 replicates consisting of 10 birds each in a completely randomized design. Basal diet was formulated to meet the nutritional requirements of broiler chicks, feed and clean water were given ad libitum throughout the experiment which lasted for 28 days. Birds in treatment 1 (T1) were fed basal diet with Oxytetracycline at 1.5g/kg feed while birds in T2, T3, T4, T5 and T6 were fed basal diet mixed with MOO at 0.1, 0.2, 0.3, 0.4 and 0.5 mL/kg feed respectively. Results on gas chromatography- mass spectrometry (GC-MS) revealed the presence of 17 bioactive compounds which accounted for 70.72 %. The major compounds identified in MOO are: β-caryphyllene (19.02 %), β-myrcene (16.08 %), carvenone (10.11 %) and α-cubebene (7.11 %) respectively. Data on average daily weight gain, average daily feed intake and feed conversion ratio were not significantly (P<0.05) different among the treatments. Highest mortality was recorded among birds in T1 (0.33 %) followed by T2 (0.01 %) none was recorded in the other treatments (P<0.05). Results on nutrient retention (dry matter, crude protein, crude fibre, ether extracts and nitrogen free extracts) were influenced by the dietary inclusion of MOO. It can be concluded that MOO could be fed to broilers up to 0.5 mL per kg feed without causing any negative effect on the performance of birds.

Keywords: *Moringa oleifera oil, broilers, antibiotics, phytochemicals, gas chromatography*

Introduction

Antibiotics have been used as growth promoting substance. The mode of action of antibiotics is that they alter microbial metabolism thereby suppressing the growth of pathogenic microbes in the gut (Oluwafemi *et al.*, 2020, Shittu *et al.*, 2021). However, the use of antibiotics is restricted due to drug resistance, drug residue in the carcass, and also the alteration of natural gut micro flora (Agubosi *et al.*, 2021; Alagbe *et al.*, 2020). Consistent use of antibiotics will not only lead to various health issues but could also cause antimicrobial resistance as well as toxic residues in animal products (Olafadehan *et al.*, 2021; Adewale *et al.*, 2021). Recently, the use of essential oils has been found to be one of the alternatives to the use of antibiotics because it is safe and effective (Musa *et al.*, 2020; Shittu and Alagbe, 2020). Essential oils contains several bioactive chemicals or secondary metabolites which performs antimicrobial, antifungal, antibacterial, antioxidants, hepato-protective and hypolipidemic activities and are generally regarded as safe (GRAS) when used in right doses for animals (Singh *et al.*, 2021). Essential oils extracted mainly

from spices and herbs and their purified compounds have been shown to have antimicrobial actions *in vitro* (Ultee *et al*., 2002; Faleiro *et al.*, 2003).

Moringa oleifera is the best-known species of the genus Moringa, a small group of plants within the order Brassicales, a family that includes cabbage and radish along with the family of cress and capers (APG, 2009). The most closely related family to Moringaceae is Caricaceae, which includes papaya, share both the characteristic of glands at the apex of the petiole (Olson, 2002). Moringaceae comprises only one genus, Moringa. Moringa embraces 13 species; arborea, concanensis, drocanensis, drouhardii, hildebrandtii, pygmeae, pilgrim, rospolianaovalaifolia, stenopetala, rivae, oleifera, and borziana, which cover a diverse range of habits or growing ways from sorts of herbs and shrubs to large trees (Olson and Razafimandimbison, 2000; Atawodi, *et al*., 2010). While varying greatly in form, it is very easy to distinguish a member of Moringa (*Moringa oleifera*) from any other plant.

Moringa (*Moringa oleifera*) is known worldwide under several popular names such as horseradish tree, drumstick tree, "Guili gandja," "Gagawandalahai," and many others (Morton, 1991). *Moringa oleifera* Lamarck or *Moringa pterygosperma* Gaertner is a South Asian plant native to the Himalaya Mountains, from Northwest Pakistan to North India (Ramachandran, *et al*., 1980). This plant is now cultivated in all tropical and subtropical regions such as Pakistan, Arabia, Central America, North and the South Philippines, Cambodia, Caribbean Islands, and Africa (Morton, 1991; Mughal, *et al*., 1999). This is due to its resistance to different climates, poor and averagely dry soils, and the multiple properties which abound to this plant (Morton, 1991; Mughal *et al*., 1999).

Essential oil fractions from Moringa (*Moringa oleifera*) contain antimicrobial compounds which confers its ability to perform several biological activities (Wenk, 2000). *Moringa oleifera* oils are commercially available and are used extensively in medicine and in the food and cosmetic industries. In addition to their antimicrobial activity, they possess biological activities such as that of antioxidant, antimicrobial, antifungal, hepato-protective as well as hypocholesteremic functions (Mellor, 2000). *Moringa oleifera* oil provides a totally new approach to improving feed digestion. The use of moringa oils in animal production may, therefore, have a promising potential as growth promoters without the adverse effects of antibiotics.

Therefore this experiment was carried out to determine effect of dietary inclusion levels of *Moringa olerifera* oil on the growth performance and nutrient retention of broiler chicks.

Materials and methods

Experimental site

This study was conducted at the university of Abuja teaching and research farm, main campus, Gwagwalada, Abuja. Gwagwalada is one of the six (6) area councils of the Federal Capital Territory of Nigeria. It lies between latitude 08°51 and 09°37N and longitude of 007°20 and 007°51 E and the land mass covers 65sq km. the weather generally warm, characterized by dry season which starts between November to April. Rainfall is moderate with annual total rainfall approximately 1100mm to 1650mm with about 60% of the annual rainfall during the months of July, August and September. The highest temperature in Gwagwalada occurs during the dry season between January and April and during this period, the maximum temperature ranges between 30° to 34° C.

Collection of plant material and extraction of Moringa oil

Moringa oleiferi seed was collected within Kuje, Area Council Abuja and authenticated at the Department of Taxonomy, Sumitra Research Institute, Nassarawa where a voucher specimen was deposited with a reference number RT/MOA/2ABJ. Seed of the samples were collected from the pod of the plants and sun dried for 11 days and pulverized into powder using laboratory grinder. 1000 grams of the powdered seeds was put into a porous thimble and placed in a Soxhlet extractor (GH-2A11 model, Punjab, India) using 300 ml of n-Hexane as extracting solvent for 2 hours until the required quantity was obtained. The oil was obtained after evaporation using a water bath at 70**°C** to remove the excess solvent from the extracted oil. The oil was kept in a well labeled container for further analysis.

Gas chromatography- mass spectrometry (GC-MS) analysis

The essential oil of *Moringa oleifera* was subjected to GC-MS using Shimadzu GC-MS (Model QP-2010A, China) equipped with an Elite-I fused silica capillary column (30m × 0.25 mm × ID × 1µm). Injection temperature was maintained at 25°C, helium flow rate as 1.5ml/min and ion source temperature at 230°C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Identification of bioactive compounds

Identifications of the compounds were based on mass spectral matching with standard compounds in National Institute of Standard and Technology (NIST) having more than 62000 patterns.

Experimental birds and their management

A total number of 180 1-day-old (Arbor acre) broilers chicks of mixed sex were used for the experiment. The chicks were randomly allotted into six (6) dietary treatments replicated thrice. Birds were raised in a battery cage system with standard dimensions of (220 ×105× 95 cm) (L×B×H). The batteries were suspended 100 cm above floor level in a well-ventilated pen. Brooding was done for a period of three weeks with the aid of 200 watts bulb fixed in each cage. Vaccines and drugs were administered according to prevailing disease condition in the environment and all other routine management was strictly adhered to. Feed and fresh water were supplied *ad-libitum* to birds throughout a period of 4 weeks of the experiment.

Experimental design and diet

The completely randomized designed (CRD) was used. Birds in treatment 1 (T1) was given the basal diet with Oxytetracycline at 1.5 g/kg feed while T2, T3, T4, T5 and T6 were fed basal diet with *Moringa oleifera* oil at 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5 mL/kg of feed respectively. Basal diet was formulated to meet the nutritional requirements of the broiler starter chicks as recommended by Aduku, (1994) as shown in Table 1.

Data collected

Feed intake (g) = Feed supplied (g) – left over (g)

Weight gain (g) = final weight (g) – initial weight (g)

Average daily gain (ADG) = Final body weight – Initial body weight
Total days of the experiment

Average daily feed intake (ADFI) = Total feed intake

Total days of the experiment

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g)

Mortality = Number of dead birds/replicate × 100
Total number of birds in replicate

Nutrient retention trial

A nutrient retention trial was carried out on the 4th week of the experiment; two birds were selected from each replicate making a total of ten (12) birds per treatment. The birds were housed in a battery cage constructed with metal trays for fecal collection. The birds were given a known amount of feed for three days and clean water was also given throughout the experiment. Feed consumed was measured by weighing the left over feed daily and subtracting from amount of feed provided. Excreta was collected for 5 days, dried and mixed thoroughly. Contaminants were carefully removed and the excreta were stored in containers. Samples were subsequently oven dried at 80^{o}C and taken for proximate composition in the laboratory using the methods described by Association of Analytical Chemist (AOAC, 2000).

Statistical analysis

Data collected on performance and nutrient retention were subjected to analysis of variance (ANOVA) using SAS statistical package, SAS (2000). The means were separated using Duncan multiple range test of the same software.

Table 1: Ingredient composition of the experimental diets

Ingredients	Quantity
Maize	54.56
Wheat offal	1.10
Soya bean meal	11.00
Groundnut cake	25.23
Fish meal (72%)	3.00
Limestone	1.50
Bone meal	3.00
Lysine	0.24
Methionine	0.21
*Premix	0.25
Salt	0.30
Toxin binder	0.10
Total	100.0
Calculated analysis (% DM**)**	
Crude protein	23.08
Crude fibre	3.00
Ether extract	4.03
Calcium	1.80

Phosphorus	0.98
Energy (Kcal/kg)	2911.3

*****Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg

Table 2: GC-MS analysis of *Moringa oleifera* oil (MOO)

S/N	Compounds	Area (%)	Retention time (min)
1	β-Myrcene	16.08	4.18
2	β-caryphyllene	19.02	11.60
3	3-terpinene	0.26	6.55
4	Isoterpinolene	0.40	1.46
5	Gallic acid	10.67	9.42
6	Carvenone	10.11	11.40
7	β-santalene	1.20	1.44
8	α-cubebene	7.11	12.38
9	Hexane	0.34	15.80
10	Ethylgallate	1.22	11.57
11	α-longipinene	0.51	15.21
12	Terpinen-4-ol	0.01	13.04
13	α-pinene	1.71	7.06
14	γ-terpinene	0.14	7.55
15	γ-eudesmol	1.60	9.10
16	Protocatechuic acid	0.27	9.55
17	Torreyol-α-cadinol	0.07	5.80
	Total	70.72	

Table 3: Effect of dietary inclusion levels of *Moringa oleifera* oil on performance of chicks

Parameters	T1	T2	T3	T4	T5	T6	SEM	SIG
IBW(g)	39.67	39.98	39.99	39.97	39.98	39.99	0.30	NS
FBW (g)	525.93c	622.2b	635.6b	704.0a	709.4a	720.4a	15.5	*
WG (g)	486.26c	582.2b	595.6b	664.0a	669.0a	680.4a	15.5	*
ADWG (g)	17.37c	20.29b	21.27b	23.71a	23.89a	24.00a	0.61	*
TFI (g)	1100.7c	1125.9ab	1134.1a	1129.3ab	1124.1b	1108.4c	4.62	*
ADFI (g)	39.31c	40.2ab	40.5a	40.3ab	40.1b	39.6c	0.21	*
FCR	2.2a	1.93ab	1.90ab	1.70c	1.70c	1.63c	0.11	*
Mortality (%)	0.33a	0.01b	-	-	-	-	0.01	*

IBW: Initial body weight; FBW: final body weight; WG: weight gain, ADWG: average daily weight gain; TFI: total feed intake, ADFI: average daily feed intake, FCR: feed conversion ratio;

[a, b, c:] Means with different superscripts within the same row differ significantly ($P < 0.05$); T1: basal diet + 1.5 g/kg Oxytetracycline; T1: basal diet + 0.1mL/kg diet; T2: basal diet + 0.2mL/kg diet; T3: basal diet + 0.3 mL/kg diet; T4: basal diet + 0.4 mL/kg diet; T5: basal diet + 0.5 mL/kg diet

Table 4: Effect of dietary inclusion levels of MOO on nutrient retention of broiler chicks

Parameters	T1	T2	T3	T4	T5	T6	SEM	SIG
DM (%)	79.90c	80.17ab	81.12ab	86.20a	87.01a	87.40a	0.42	*
CP (%)	73.72d	75.40c	77.86ab	76.60bc	78.09a	78.44a	0.69	*
CF (%)	42.07d	40.17c	45.60c	48.60ab	49.62a	46.09cb	1.49	*
EE (%)	59.80d	60.02cd	60.00cd	60.55b	60.28bc	61.80a	0.21	*
NFE (%)	87.60b	89.40a	89.49a	89.00ab	89.50a	89.90a	0.74	*

[a, b, c, d:] Means with different superscripts within the same row differ significantly ($P < 0.05$); DM: Dry Matter; CP: Crude Protein, CF: Crude Fiber, EE: Ether Extract, NFE: Nitrogen Free Extract, SEM: Standard Error of Mean; [a, b, c:] Means with different superscripts within the same row differ significantly ($P < 0.05$); T1: basal diet + 1.5 g/kg Oxytetracycline; T1: basal diet + 0.1mL/kg diet; T2: basal diet + 0.2mL/kg diet; T3: basal diet + 0.3 mL/kg diet; T4: basal diet + 0.4 mL/kg diet; T5: basal diet + 0.5 mL/kg diet

Results and discussion

GC-MS analysis of *Moringa oleifera* oil (MOO)

GC-MS analysis of *Moringa oleifera* oil (MOO) is presented in Table 2. 17 bioactive compounds were identified which accounted for 70.72 % of the oil. β-Myrcene (16.08 %), β-caryphyllene (19.02 %), 3-terpinene (0.26 %), isoterpinolene (0.40 %), gallic acid (10.67 %), carvenone (10.11 %), β-santalene (1.20 %), α-cubebene (7.11 %), hexane (0.34 %), ethylgallate (1.22 %), α-longipinene (0.51 %), terpinen-4-ol (0.01 %), α-pinene (1.71 %), γ-terpinene (0.14 %), γ-eudesmol (1.60 %), protocatechuic acid (0.27 %) and torreyol-α-cadinol (0.07 %). β-caryphyllene has the highest concentration in MOO is a bi-cyclic hydrocarbon sesquiterpene which are therapeutically significant plant substances commonly found to have anti-inflammatory and antioxidant properties (Chaitanya *et al.*, 2021; Shittu and Alagbe, 2020). Gallic acid is group of flavonoids known to possess antibacterial, antioxidants and antiviral activities (Faizi *et al.*, 2003). Flavonoids have been shown to have antifungal activity in vitro studies (Galeotti *et al.*, 2008). The potent antioxidant activity of flavonoids reveals their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals; this may be the most important function of flavonoids (Chen *et al.*, 2000). They also induce mechanisms that may kill cancer cells and inhibit tumor

invasion (Williams *et al*., 2004). The flavonoids present may be responsible for the medicinal properties accorded the plant (Saleem *et al*., 2005; Alagbe, 2022). α-pinene, γ-terpinene, γ-eudesmol, protocatechuic acid and torreyol-α-cadinol also have high therapeutic value and can function as antimicrobial, anticarcinogenic and anti-diuretic (Sittanikove *et al.*, 2001). Phenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and protein that play a role in chronic disease, (Ojewuyi *et al*., 2014).

Effect of dietary inclusion levels of *Moringa oleifera* oil on performance of chicks

Effect of dietary inclusion levels of *Moringa oleifera* oil on performance of chicks is presented in Table 3. Final body weight (525.93 – 720.4 g), weight gain (469.3 – 680.4 g), average daily weight gain (ADWG) (17.37 – 24.00 g), total feed intake (TFI) (1100.7 – 1134.1 g), average daily feed intake (39.31 – 40.50 g), feed conversion ratio (1.63 – 2.2) and mortality (0.01 – 0.33 %). ADWG, ADFI and feed conversion ratio and mortality values were significantly ($P<0.05$) different among the treatments. Highest mortality was recorded in T1 (0.33 %) followed by T2 (0.01 %) none were recorded in the other treatments ($P<0.05$). The improved ADWG and AFFI in T3, T4 and T5 could be due to the phytochemical or bioactive constituents in *Moringa oleifera* oil. Phytochemicals in some plant extracts enable them to perform multiple biological roles such as antimicrobial, antioxidant, anti-inflammatory, antifungal, antiviral and hepato-protective activities (Alagbe *et al*., 2020; Kalemba *et al*., 2003; Agubosi *et al*., 2022). The observation is consistent with the reports of previous researchers (Zhang *et al*., 2005; Lee *et al*., 2003). Low mortality observed in birds fed different inclusion of MOO is an indication that it can potentially minimize the occurrence of intestinal diseases caused by pathogenic microorganisms and could favour the growth of beneficial gut microbiota supporting growth and promoting the immune system (Bento *et al*., 2013; Lopez *et al*., 2007). Kalemba and Kanicka (2003) also reported that phytochemicals are capable of improving the palatability and retention time of feeds but contrary to the findings of Amad *et al*. (2011) who recorded a numerical decrease in average daily feed intake of broilers fed a blend of thyme, star anise, and oregano leaves, and its associated essential oils compared with control diet. The reduced in feed intake could be attributed to unpleasant smell which rendered the diet unpalatable to the birds.

Effect of dietary inclusion levels of MOO on nutrient retention of broiler chicks

Table 4 shows the effect of dietary inclusion levels of MOO on nutrient retention of broiler chicks. The proximate components revealed the presence of dry matter (79.90 – 87.40 %), crude protein (73.72 – 78.44 %), crude fibre (40.17 – 49.62 %), ether extract (59.80 – 61.80 %) and nitrogen free extract (87.60 – 89.90 %). Dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and nitrogen free extract values were significantly ($P<0.05$) different among the treatments. Maximum DM values were recorded among birds in T3, T4 and T5, intermediate in T2 and minimum in T1 ($P<0.05$). The higher DM is an indication that MOO has beneficial effects on nutrient utilization possibly by stimulating digestive enzymes such as lipase, amylase, or protease (Platel and Srinivasan, 2004) and improves gastrointestinal morphology (Jamroz *et al*., 2006; Upadhaya *et al*., 2016 b). According to Hafeez *et al*. (2003), dietary inclusion of thyme essential oil at 100 mg /kg of feed improved performance and apparent ideal digestibility of nutrients compared

with control in broiler chickens. Similar result was recorded by Emami *et al*. (2012) who observed that broiler fed peppermint oil at 200 mg/kg feed led to the increase of crude protein digestibility. The improvement of nutrient retention of broiler chicks given different levels of Moringa oil could probably due to stability and increase in beneficial bacteria in the intestinal flora.

Conclusion

It can be concluded that MOO is loaded with several bioactive compounds which are capable of performing multiple biological activities and it could be fed to broilers up to 0.5 mL per kg feed without causing any negative effect on the performance of birds.

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